

iGEM TU/e 2014

Biomedical Engineering

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Colony PCR

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1 Colony picking

- Using a pipet pick a number (4-6) colonies and put them into an ept with 15 μ L H₂O.

2 PCR

- Construct the PCR mixture in the following way:

Component	Quantity/mass/final concentration	Volume (μ L)
DNA (from bacteria)	<i>Pipette tip in 15 μL H₂O</i>	1
2x KAPA2G mix	1x	12.5
Primer FW	0.5 μ M (10 μ M)	
Primer RV	0.5 μ M (10 μ M)	
H ₂ O		9
<i>Total</i>		25

Total mastermix

Component	Quantity/mass/final concentration	Volume (μ L)
DNA (from bacteria)	<i>Pipette tip in 15 μL H₂O</i>	1 for each separate PCR mixture
2x KAPA2G mix	1x	62.5
Primer FW	0.5 μ M (10 μ M)	
Primer RV	0.5 μ M (10 μ M)	
H ₂ O		45
<i>Total</i>		125

- Run PCR in the PCR machine with the following protocol:

Step	Temperature ($^{\circ}$ C)	Time (sec)	Cycles
Denaturation	95	180 (3 min)	1
Denaturation	95	15	35
Annealing	55-60	15	
Extension	72	20 sec/kb	
Final extension	72	600 (10 min)	1
Cooling	4	Hold	1

3 Gel electrophoresis

- Once the colony PCR has been completed the samples (20 μ L; first mix with 4 μ L loading dye) should be loaded on to an electrophoresis gel and run.

After colony PCR do a plasmid amplification of the vectors that are correct.